

Stillbirth following severe symmetric fetal growth restriction due to reactivation of Epstein–Barr virus infection in pregnancy

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Abstract

Epstein–Barr virus (EBV) infection in pregnancy and consequent fetal outcomes are rarely reported. The majority of cases described strongly support the possibility of transmission of this virus in utero and during delivery, resulting in stillbirth and/or congenital defects. We present a case of EBV reactivation in pregnancy that caused a severe symmetrical fetal growth restriction (FGR) and ultimately spontaneous fetal death. A 36-year-old woman, whose infection status was undetermined, was diagnosed with severe FGR at 24 weeks' gestation. The fetal karyotype was normal. EBV DNA was detected in the amniotic fluid and maternal immunoglobulin G antibodies were positive. At 30 weeks' gestation, the fetus died spontaneously. Placental examination found evidence of deciduitis and villitis. Reactivation of EBV infection appears to be related to FGR and warrants further research to determine the optimal management strategy in pregnancy.

Key words: Epstein–Barr virus infection, fetal growth retardation, reactivation of Epstein–Barr virus infection.

Introduction

Epstein–Barr virus (EBV) is a herpes virus that infects more than 90% of asymptomatic people.¹ If the individual is infected during adolescence and adulthood, EBV can cause a benign lymphoproliferative disease known as infectious mononucleosis.² In pregnancy, similarly to cytomegalovirus (CMV), varicella zoster virus and human herpes virus (HHV) types 6, 7 and 8, EBV shares the ability to establish latency, but can become reactive at a later time.³ Structural fetal abnormalities can result from intrauterine infection and transmission of the infection during pregnancy, and transmission by blood at the time of delivery may result in neonatal disease.³

However, EBV-related pregnancy outcome data is conflicting regarding adverse outcomes: according to Eskild *et al.*, while pregnancy is a state of altered immu-

nity, it is not associated with increased EBV reactivation, and thus there is no association between EBV antibody status and fetal death.⁴ Haeri *et al.* reported on EBV reactivation as a biological marker of stress in pregnancy, since there is mounting evidence that stress promotes latent EBV reactivation in non-pregnant adult populations (with a rate of 20–30%).⁵ In addition, although cases of birth defects, prematurity and low birth weight have been observed, they could not be definitively linked to EBV infection.² Case reports and studies of EBV in amniotic fluid suggest that in utero infection does indeed occur.^{2,6} A study by Meyohas *et al.* determined that a significant reactivation of EBV infection in the first part of pregnancy was associated with earlier delivery and lower birth weight in still-born and live-born children.²

This case report of the presence of EBV in amniotic fluid, symmetric fetal growth restriction (FGR) and

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fetal demise, gives a further example of the consequences of EBV in pregnancy. In the light of the published research described above, this report indicates the need for further investigation into the consequences of maternal EBV infection during pregnancy.

Materials and Methods

A quantitative real-time polymerase chain reaction (PCR) (Bio-Rad Laboratories, Philadelphia, PA, USA) was applied for amniotic fluid. A multicolor real-time PCR has been used with the following fluorophores containing DNA probes (TaqMan probe; IDT, San Jose, CA, USA):

- Fluorophore containing Fam for detecting EBV, toxoplasmosis, parvovirus B19, adenovirus and treponema DNA
- Fluorophore containing Hex for detecting CMV DNA
- Fluorophore containing Cy5 for detecting HHV DNA.

We quantified the number of copies of DNA virus based on three standard levels: S1, 10 000 copies/mL; S2, 1000 copies/mL; and S3, 100 copies/mL.

Case Report

A 36-year-old woman, gravida 2, para 1, attended routine follow up for her pregnancy at 24 weeks' gestation, at which time the fetus was diagnosed with FGR. The obstetric history of this pregnant woman was unremarkable with no abortion, fetal and/or child

malformation. Her first daughter had been delivered in 2005 by cesarean section at term. There was no evidence of infection in the first trimester, confirmed by laboratory tests, which were negative for toxoplasmosis, CMV, rubella, hepatitis B virus, human immunodeficiency virus (HIV) and syphilis.

Fetal development was normal until the 24th week of gestation: the fetal nuchal translucency thickness was 1.1 mm (crown rump length = 50 mm) and the possibilities of trisomy 21 and 18 were calculated using a combined test as 1/398 and 1/20 176, respectively. At the 24th week of gestation, routine morphological scanning showed the presence of hyperechoic bowel (grade 2) and symmetrical FGR. Doppler velocimetry of the uterine artery was normal, there was no notch sign (resistance index [RI] = 0.55 in left uterine artery and RI = 0.47 in the right uterine artery) (Fig. 1), and an absence of end-diastolic frequencies was diagnosed in the umbilical artery (Fig. 2). Doppler velocimetry of the middle cerebral artery and ductus venosus were normal (Figs 3,4).

The patient and her husband gave their consent for amniocentesis to diagnose fetal chromosomal aberrations. The fetal karyotype was confirmed to be normal (Fig. 5). Toxoplasmosis, parvovirus, adenovirus, treponema, CMV, HHV 1 and 2, and EBV were routinely screened by using antibody measurements in the maternal blood and real-time PCR in the amniotic fluid. EBV was incidentally detected in the amniotic fluid, with 26 800 copies/mL. The patient was then further tested for EBV, and it was demonstrated to be present in her peripheral blood: immunoglobulin (Ig)G antibodies to the EBV capsid antigen were

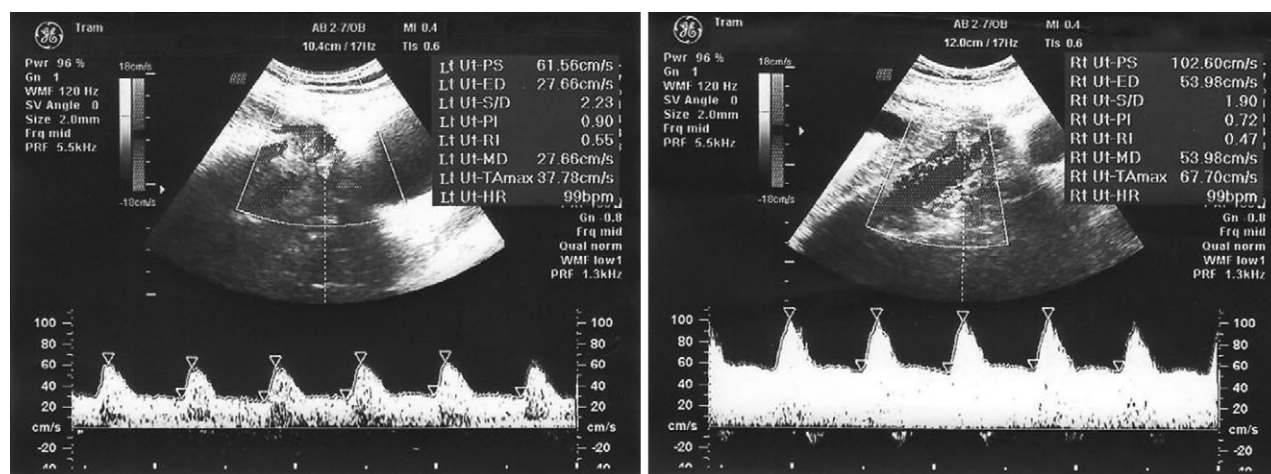


Figure 1 Normal left and right uterine arteries at 24 weeks' gestation (no notch sign).



Figure 2 Absent end-diastolic frequencies in the umbilical artery. This sign was consistent from the 24th to the 30th weeks of pregnancy.

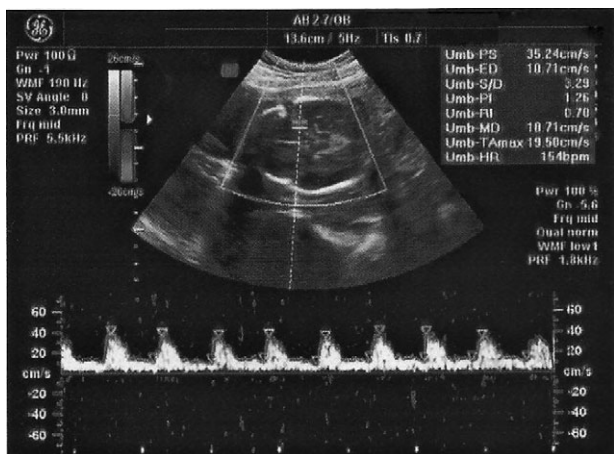


Figure 3 Normal Doppler velocimetry of the middle cerebral artery at 24 weeks' gestation (no notch sign, normal pulsatility index [PI] and resistance index [RI]).

positive (174 IU/mL), and IgM antibodies were negative.

After informed consent, the patient and her husband preferred to pursue the pregnancy for as long as possible. Fetal growth was reviewed every two weeks by measurement of biparietal diameter (BPD), head circumference (HC), femur length (FL) and abdominal circumference (AC). All fetal biometric parameters were consistently found to be lower than the third percentile line (Fig. 6). Doppler velocimetry of the umbilical artery, the middle cerebral artery, and the ductus venos were performed routinely every two weeks. There was a consistent

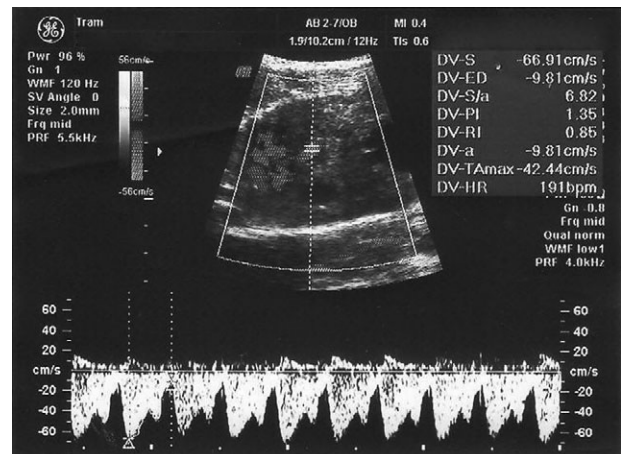


Figure 4 Normal Doppler velocimetry of the ductus venos at 24 weeks' gestation (normal a-wave, normal pulsatility index [PI] and resistance index [RI]).

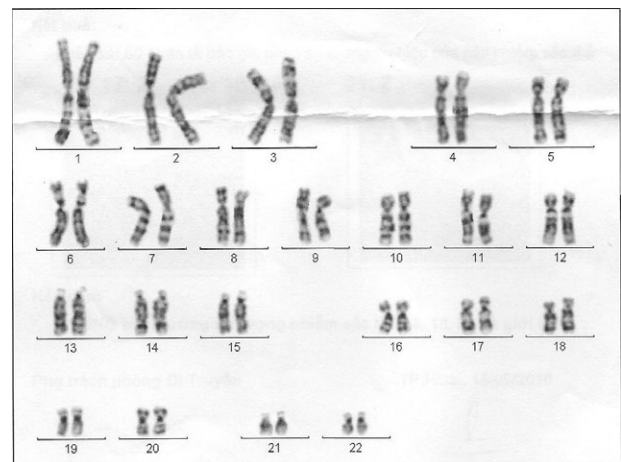


Figure 5 Normal fetal karyotype (46, XX).

absence of end-diastolic frequencies in the umbilical artery, the pulsatility index (PI) of middle cerebral artery was found to be below the third percentile (Fig. 7) and the ductus venos continued to show a normal a-wave.

At the 30th week of gestation, the fetus demised spontaneously in utero, and after an induced labor, the patient delivered a female infant transvaginally. The stillborn baby weighed 900 grams and the placenta was small, weighing 200 grams. Histopathological examination of placental tissues demonstrated deciduitis and villitis with a significant mononucleocyte infiltration, trophoblastic necrosis and capillary endothelial damage. The couple refused an autopsy for religious

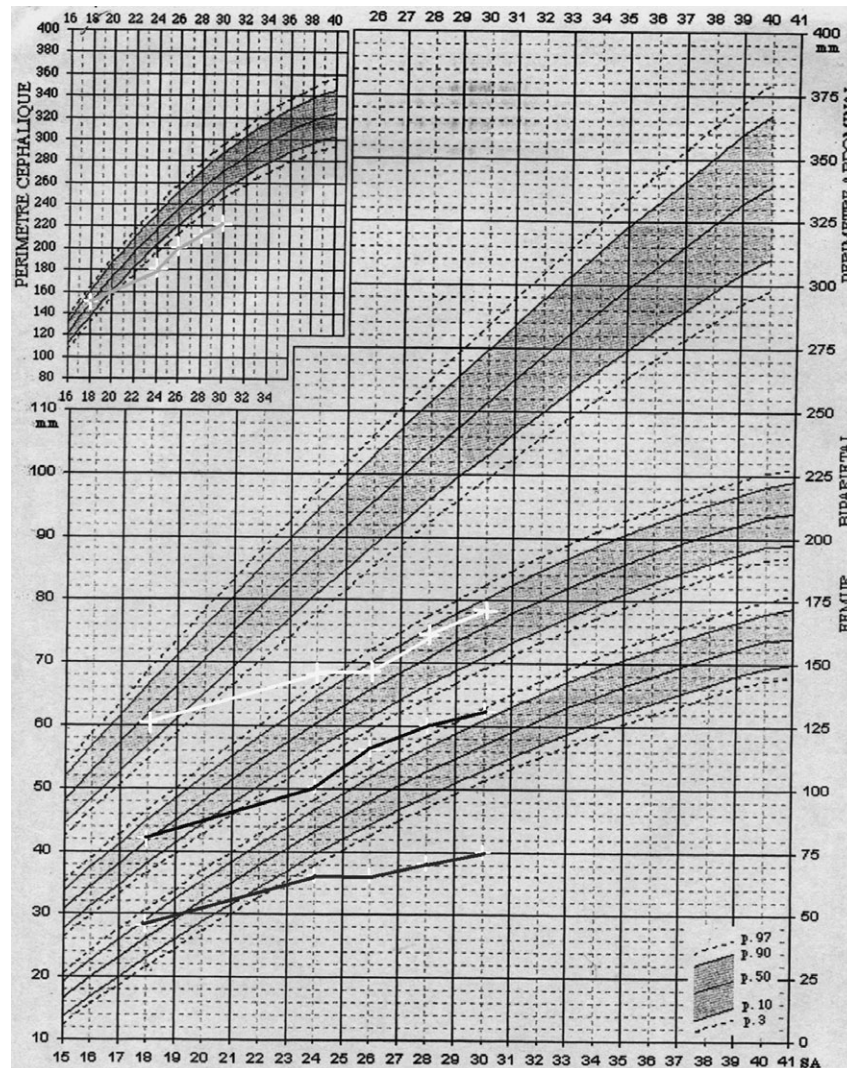


Figure 6 Development of fetal biometry (biparietal diameter, head circumference, femur length and abdominal circumference) from the 18th to the 30th weeks of gestation. All parameters were found to be below the third percentile line.

reasons, further histopathological and immunohistochemical testing is not currently possible in our resource-limited setting.

Discussion

This case report demonstrates symmetrical FGR and early intrauterine death as apparent consequences of reactivation of EBV infection in pregnancy. There is very little known about maternal EBV infection and pregnancy outcomes, since most published material are case reports: some reported cases suggest a relationship between EBV and congenital heart disease,^{7,8,9}

and FGR and multiple congenital anomalies;^{10,11} others support the concept that entry of a viral genome into the amniotic fluid occurred in normal pregnancies without any perinatal impacts.¹² With evidence of a poor fetal outcome and proven EBV infection in pregnancy, we think that EBV infection during pregnancy may indeed produce an effect on perinatal and neonatal outcomes.

Eskild *et al.* consider that adverse pregnancy outcomes are not associated with EBV reactivation.⁴ Despite this conclusion, evidence has accumulated in the last few years highlighting the complexity of the interaction between EBV and the human host.¹³ The

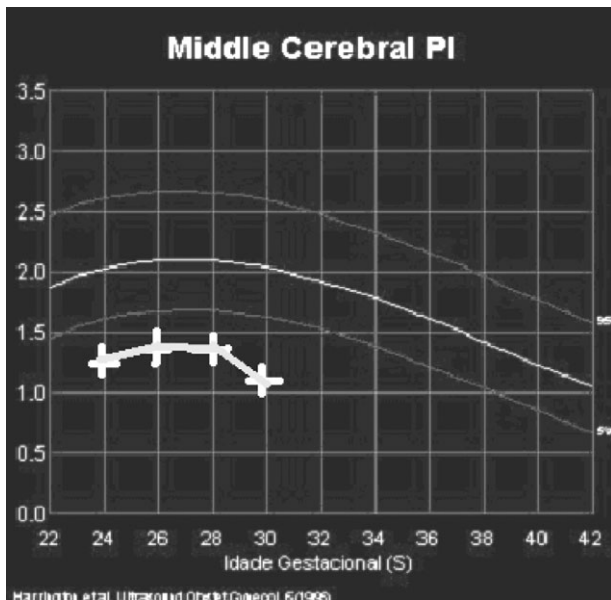


Figure 7 Abnormal pulsatility index (PI) curve of the middle cerebral artery.

actual damage to the developing embryo or fetus from maternal herpes simplex virus and EBV appears to be very small.^{14,15} But both herpes simplex virus and EBV seem to be able to cross the placenta and cause, as described by several investigators, placental infection manifested by deciduitis and villitis. These placental changes potentially cause fetal damage: Brown and Stenchever reported on an infant exposed to EBV from conception to delivery: the infant was delivered at 42 weeks' gestation weighing 1720 g, showing symmetrical growth retardation and died several minutes after delivery; post-mortem examination revealed multiple anomalies of the craniofacial complex, kidneys, lungs, heart and brain.⁷ This case demonstrates that EBV may cross the placenta causing placental and fetal infection.^{10,15}

In our case report, reactivation of EBV infection during pregnancy was suggested because of a presence of a high titer of IgG antibodies in maternal peripheral blood (17 times higher than our laboratory cut-off [10 IU/mL]) and real-time PCR evidence of EBV DNA in the amniotic fluid. In addition, the IgM antibody titer was negative, so there was no evidence of a primary infection. We propose that the impact of EBV was to disrupt placental development, which then caused a symmetrical FGR and an absence of end-diastolic frequencies in the umbilical artery, and finally fetal demise. We are limited in not being able to give

more certain evidence of EBV reactivation through histopathology and immunohistochemistry testing of the placenta; however, this case is consistent with the study of Eskild *et al.*, who demonstrated that a reactivation of EBV infection is associated with lower birth weight in stillborn and live-born children.⁴

There is no published evidence in the literature that EBV has an effect on the developing embryo or fetus.^{5,15,16} Similarly, in this case, we found that the fetal karyotype was normal (46, XX) and no fetal structural abnormality was detected on ultrasonography. We also conclude that EBV reactivation during pregnancy does not represent a major teratogenic risk to the fetus as Miller *et al.* affirmed.¹²

However, there is a need for further studies to verify the actual impact of EBV exposure on the fetus itself during pregnancy. In the majority of the cases describing the influence of maternal EBV infection on pregnancy outcome, there seems to be placental, cardiac and visual involvement.^{7,8,10,11,15,16} Avgil *et al.* found two cases of congenital heart anomalies (ventricular septal defect and persistent foramen ovale) in women who had a recurrent EBV infection during the first trimester of pregnancy.¹⁶ They ruled out the possible association of congenital heart disease and an exposure to EBV from conception to delivery.¹⁶ Recently, Werler *et al.* demonstrated that EBV reactivation resulting from multiple challenges to the immune response, such as pregnancy, age and toxic exposure might be related to a risk of gastroschisis.¹⁷ Therefore, they propose further research into gastroschisis caused by EBV and other herpes viruses. The role of EBV in pregnancy, according to Haeri *et al.*, should be examined.⁵

Although reports of pregnant women with EBV are rare,¹⁵ EBV infection in pregnancy does seem to have a close relationship with stillbirth and congenital defects.¹⁰ Our results support the studies that demonstrate an association between EBV reactivation and placental damage during pregnancy.^{10,11,13,16} We do not recommend adding EBV testing in the universal TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes simplex and HIV) screening for infection during pregnancy, because the seroprevalence of this virus is very rare in this population. Our message in this case report is that the obstetricians should be aware of the need for testing EBV infection during specific pregnancies when trying to elucidate the possible cause of early fetal growth restriction and/or intrauterine death. We acknowledge that these findings need confirmation through future studies for prognosis of pregnancy with EBV infection.

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